Associative Rhizosphere Nitrogen Fixation (Acetylene Reduction) Among Plants from Ohio Peatlands

Conrad E. Wickstrom and Ralph J. Garono[1], Department of Biological Sciences, Kent State University, Kent, OH 44242

Abstract. The macrophytes of wetlands are known, and in some cases, the nitrogenase activity (NA) of these plants has been examined as well. This activity, however, has been only partially studied in peatlands, particularly in northeastern Ohio where numerous peat-forming bogs and fens are located. We tested 18 flowering plants and 1 Bryophyte inhabiting peatlands in this region of the United States for potential rhizosphere nitrogen fixation using the acetylene reduction technique. The roots and adherent soil of selected plants from 3 bogs (Fern Lake, Kent, and Triangle Lake) and 1 fen (J. Arthur Herrick) were tested for ethylene evolution following incubation under an acetylene atmosphere. NA was observed at each peatland, and 89% of the plants tested were NA positive. The signature plants of the bogs, *Chamaedaphne calyculata*, and the fen, *Potentilla fruticosa*, were NA positive, whereas *Sphagnum* spp. was negative at 2 of the 4 peatlands. The highest rates of NA were associated with *Typha* spp. where the mean rates for ethylene evolved by *Typha angustifolia* rhizosphere were 233.2 nmoles g Dry Mass^{-1} 24 h^{-1} and 407.2 X 10^{2} nmoles m^{-2} 24 h^{-1}. Rhizosphere NA of 3 previously unstudied plants (*Acorus americanus*, *Decodon verticillatus* and *Symplocarpus foetidus*) are given. Diazotroph relationships directly and indirectly enrich the associated plants and soil with nitrogen such that the success of newly constructed or reclaimed wetlands may be fostered by inclusion of plant species known to harbor associative diazotrophs. The widespread occurrence and frequency of nitrogen fixation in these habitats argues for inclusion of this activity during investigative and management studies of wetlands.

OHIO J SCI 107 (3): 39-43, 2007

INTRODUCTION

Prokaryotes with nitrogenase activity (NA) and capable of nitrogen fixation are often found in association with plants (Evans and Burris, 1992). Certain species form mutualistic associations in actinorhizal (Baker and Mullin, 1992; Del Tredici, 1995) and rhizobial (Elkan and Bunn, 1992) root nodules; other diazotrophs in less species-specific endophytic relationships are known (Baldani and others 2000). Additionally, free-living diazotrophs may establish more loosely organized protocooperative associative relationships in the rhizoplane and rhizosphere of higher plant roots (Biesboer, 1984; Klucas, 1991; Elmerich and others, 1992).

Associative diazotrophs of the latter variety have been documented for pelagic as well as littoral macrophytes in ponds and larger bodies of freshwater (Wickstrom and Corkran, 1997). Plant species populating the margins of these lentic systems also support diazotrophs, and the rooted plants of marshes and other wetlands are no exception (Waughman and Bellamy, 1980). Both narrow- and broad-leaved cattails support rhizosphere NA (Eckardt and Biesboer, 1988a). In addition to *Typha* spp., Eckardt and Biesboer (1988b) reported nitrogen fixation for other wetland species such as have Tjepkema and Evans (1976), Kana and Tjepkema (1978), Ogan (1982), and Quale (1994).

Northeastern Ohio is home to a large array of permanently waterlogged, peat-forming wetlands such as bogs and fens (Denny, 1979; Andreas, 1985). While the flora of these peatlands has been documented (Andreas and Knoop, 1992), the occurrence of diazotrophic activity associated with these species has not. The purpose of this study was to document the occurrence of rhizosphere nitrogen fixation within these sites and determine the frequency and levels of nitrogenase activity among the plants growing there. Toward this end, we studied 4 disjunct peatlands and tested 19 species of their plant inhabitants for rhizosphere acetylene-reduction activity.

MATERIALS AND METHODS

Three of the 4 sites examined, Kent Bog and Triangle Lake Bog (Portage County) and Fern Lake Bog (Geauga County), are *Sphagnum*-dominated, kettle hole lake peatlands. Andreas and Bryan (1990) detailed the plant communities of Fern Lake Bog and Triangle Lake Bog. The fourth site, J. Arthur Herrick Fen (Portage County), is associated with a glacial kame. These sites are officially recognized as bogs or fen (Andreas, 1985) with their signature indigenous flora, including *Potentilla fruticosa* L. (shrubby cinquefoil) in the fen and *Chamaedaphne calyculata* (L.) Moench in the bogs, and were selected for study because of their unique floral communities, location, and their status as State of Ohio Research Reserves. Plant identification and nomenclature follow that of Voss (1972).

Root-soil samples were collected from ca. 15 cm^{2} areas populated by the selected species. Roots were removed and placed into either a 30 cc incubation serum bottle or a Whirl-Pak™ (NASCO International Inc.) bag in the field and immediately returned to the lab where sample processing was completed. We refer to the material tested as rhizosphere, not roots, because we did not wash the roots but simply removed the loose soil. Since most of these plants are not nodulated the associated NA is attributed to diazotrophic microbes located in the rhizosphere of the root either on the rhizoplane, in the adherent soil, or endophytic in the root tissues.

Samples were incubated under a 20% CaC_2-generated acetylene gas atmosphere in the dark at room temperature (approx. 23°C). Preliminary trials with leatherleaf to optimize incubation conditions showed that ethylene evolution was not stimulated by addition of water or sparging with nitrogen gas to promote anoxia prior to introduction of acetylene. However, we found that test sensitivity was enhanced during extended incubations; therefore, we incubated most samples for 24 h.

[1]Corresponding author: Dr. Ralph J. Garono, Wetland & Watershed Assessment Group, Earth Design Consultants, Inc., 230 SW Third St., Suite 212, Corvallis, OR 97333. Phone: 541-757-7896. FAX: 541-757-7991. E-mail: rgarono@earthdesign.com.

Ethylene production was quantified by isothermal FID gas chromatography (Capone, 1993; Wickstrom and Corkran, 1997), and rhizosphere samples were oven dried at 60 °C after incubation. The amount (nmoles) of ethylene evolved per 24 h were calculated for each species. The data were then normalized for rhizosphere dry mass (DM) and for area (m^2). The 24 h period included an initial incubation lag phase, hence, the calculated nitrogenase activities are not considered steady state rate estimates. Control incubations demonstrated that ethylene was not liberated from the rhizosphere without the addition of acetylene.

RESULTS AND DISCUSSION

A total of 18 flowering plants and *Sphagnum* moss from the 4 peatlands were tested for rhizosphere nitrogenase activity (Table 1). The rhizospheres of 89% of the species tested were positive for nitrogenase activity (acetylene-dependent ethylene evolution). All plants tested at Triangle Lake Bog possessed nitrogenase activity (NA), whereas 85 and 80% of the tested Herrick Fen and Fern Lake Bog plants, respectively, were active. Only 2 of the 5 from Kent Bog were NA positive.

The rhizospheres of several species examined at multiple sites were NA positive regardless of habitat: *Carex lacustris*, *Chamaedaphne calyculata*, *Typha angustifolia*, and *Vaccinium macrocarpon* (Table 1). *Sphagnum* spp., the only plant tested at all 4 sites, was active in half of them. The literature on this genus also is variable; Koerselman and others (1989) did not observe *Sphagnum* NA, whereas Sheridan (1991) reports associated cyanobacterial NA. Although found in 3 of the peatlands, *V. corymbosum* NA was only detected at Triangle Bog. Eleven of the remaining 13 plants tested at single sites possessed rhizospheres with NA. For some of these genera, such as *Alnus* (Walker, 1989; Beaupied and others, 1990), *Nuphar* (Wickstrom and Corkran, 1997), *Potentilla* (Kana and Tjepkema, 1978)

Table 1

Peatland flowering plants tested for rhizosphere nitrogenase activity.

Plant Family	Plant species (common name)	Site[a]
Aaraceae	*Acorus americanus* (Raf.) Raf. (sweet flag)	T+
	Symplocarpus foetidus (L.) Nutt. (skunk cabbage)	H+
Corylaceae	*Alnus rugosa* (Duroi) Sprengel (speckled alder)	H+
Cyperaceae	*Carex lacustris* Willd. (sedge)	H+,K+
	Carex stipata Muhl. (sedge)	H+
	Carex stricta Lam. (sedge)	H+
	Dulichium arundinaceum (L.) Britt. (three-way sedge)	T+
Ericaceae	*Chamaedaphne calyculata* (L.) Moench (leatherleaf)	F+,K+,T+
	Vaccinium corymbosum L. (blueberry)	F-,K-,T+
	Vaccinium macrocarpon Ait. (cranberry)	F+,T+
Lentibulariaceae	*Utricularia* sp. (bladderwort)	H+
Lythraceae	*Decodon verticillatus* (L.) Ell. (swamp loosestrife)	H+
Myricaceae	*Myrica pennsylvania* Loisel. (bayberry)	H-
Nymphaceae	*Nuphar advena* (Aiton) Aiton f. (yellow pond-lilly)	H+
Pinaceae	*Larix laricina* (DuRoi) K.Koch (tamarack)	K-
Rosaceae	*Potentilla fruticosa* L. (shrubby cinquefoil)	H+
Typhaceae	*Typha angustifolia* L. (narrow-leaved cattail)	F+,H+,T+
	Typha latifolia L. (broad-leaved cattail)	H+
Sphagnaceae[b]	*Sphagnum* spp. (sphagnum moss)	F+,H-,K-,T+

[a] Sites are: Fern Lake Bog (F), J.Arthur Herrick Fen (H), Kent Bog (K), and Triangle Lake Bog (T); presence (+) or absence (-) of nitrogenase activity (NA) is indicated.
[b]Division Bryophyta.

and *Utricularia* (Wagner and Mshigeni, 1986) nitrogenase activity is documented. Rhizosphere NA has not been reported previously for sweet flag, skunk cabbage, or swamp loosestrife (Table 1).

Of the plants without NA, tamarack rhizosphere nitrogenase activity is not reported in the literature, but species of *Myrica* nodulate with *Frankia* to become diazotrophic (Troelstra and others, 1992; Young and others, 1992). Maeda and others (1999) noted this activity for *Myrica gale* in a Japanese peatland. *M. pennsylvania* tested negative for NA in Herrick Fen; however, this species is reported to fix dinitrogen elsewhere (Dudley and others, 1996). Root nodules are not required for survival or growth of *Myrica* (Troelstra and others, 1992).

Table 2 shows the nitrogenase activities for those NA positive species minimally tested in triplicate at the indicated peatland (one of the *T. angustifolia* samples from Triangle Lake Bog was lost during processing). Twenty-four h nmol ethylene normalized for rhizosphere dry mass was greatest at Fern Lake Bog (M = 128.9 nmol C_2H_4 g^{-1} DM 24 h^{-1}), while NA per m^2 was greatest at Herrick Fen (M = 790.2 X 10^2 nmol C_2H_4 m^{-2} 24 h^{-1}). Combined with the lowest frequency of NA positive samples (40%) both activity representations also were lowest in Kent Bog: 19.1 and 45.9 X 10^2, respectively.

Not only were peatland-specific levels of rhizosphere NA different, but NA also varied between species (Table 2). For plants tested at more than one peatland, *Typha angustifolia* NA was greatest both in terms of rhizosphere mass (M = 233.2 nmol C_2H_4 g^{-1} DM 24 h^{-1}) and habitat area (M = 407.2 X 10^2 nmol C_2H_4 m^2 24 h^{-1}). The cattail activities are notable because this genus is widely distributed, and its associated activity can contribute to nitrogen cycle dynamics throughout the world (Eckardt and Biesboer, 1988a; Quale, 1994).

Table 2

Nitrogenase activity associated with the rhizosphere of bog and fen species.

Site	Plant species	n		Nitrogenase Activity[a] per g root	per m^2 (x10^2)
Fern Lake Bog	*Chamaedaphne calyculata*	3		59.0	205.5
	Sphagnum spp.	3		57.4	98.9
	Typha angustifolia	3		318.5	30.7
	Vaccinium macrocarpon	3		80.8	130.9
			Mean =	128.9	116.5
J. Arthur Herrick Fen	*Carex lacustris + C. stipata*	4		32.0	571.8
	Typha angustifolia	8		117.7	798.1
	Typha latifolia	8		100.4	1000.8
			Mean =	83.3	790.2
Kent Bog	*Carex lacustris*	3		26.7	37.1
	Chamaedaphne calyculata	3		11.4	54.7
			Mean =	19.1	45.9
Triangle Lake Bog	*Acorus calamus*	3		91.8	286.0
	Chamaedaphne calyculata	3		112.1	490.0
	Dulichium arundinaceum	3		122.3	433.5
	Sphagnum spp.	3		10.6	35.5
	Typha angustifolia	2		263.3	392.7
	Vaccinium corymbosum	3		12.0	58.8
	Vaccinium macrocarpon	3		35.4	126.3
			Mean =	92.5	260.4

[a]Mean nmol ethylene evolved over 24 h period normalized to g dry root biomass or m^2.

Carex lacustris with its dense populations and extensive roots ranked second for NA per m^2 (M = 304.5 X 10^2). All species of sedges from the 4 peatlands tested positive for NA. Associative NA has been reported for various species of *Carex* (Kana and Tjepkema, 1978; Henry and Svoboda, 1986; Quale, 1994). Mean leatherleaf rhizosphere activity at 60.8 nmol C_2H_4 g^{-1} DM 24 h^{-1} lagged a distant second behind *T. angustifolia*.

If the rhizosphere nitrogenase activities exhibited by these plants were simply a property of the peatland soils in general (Zechmeister-Boltenstern and Kinzel, 1990), the incidence of rhizosphere activity should be 100% in all of the habitats tested; this was not the case. Rather, it is likely that bacteria capable of fixing nitrogen and found free-living in soils, *e.g*., *Azospirillum*, *Bacillus*, *Enterobacter*, *Klebsiella*, *etc*. (Biesboer, 1984; Ogan, 1984, Eady, 1992; Li and MacRae, 1992) are enriched by some compound(s) released from the roots of the higher plants in these systems. Plant species consistently NA positive (*e.g*., *T. angustifolia*) may release these suitable compounds regardless of habitat, whereas release by those species variably NA positive between habitats may reflect the vagaries of environmental stimuli or edaphic factors, *e.g*., compare Kent Bog with the other peatlands surveyed (Table 1). Consequently, in most of the instances of rhizosphere activity reported here, it is likely that the nitrogen-fixing association is loose and fortuitous rather than mutualistic as in the nodulated species.

The need for nitrogen fertilization and other soil ameliorations in reclaimed or newly constructed wetlands may be lessened by inclusion of non-nodulated plant species known to support and/or promote rhizosphere NA in addition to inclusion of nodulated vascular plants (Dommergues, 1997; Franco and others, 2000). The diazotroph-harboring plants promote the growth of non-fixing plants (Miller and others, 1993) either through direct transfer of fixed nitrogen (*e.g*., Dean and Biesboer, 1986; Ekblad and Huss-Danell, 1995; Uselman and others, 1999), through serving as a diazotroph inoculum capable of initiating additional associative relationships (*e.g*., Ronkko and others, 1993), or through soil modification and facilitation of indigenous or successional species *via* annual soluble exudate and particulate enrichments (*e.g*., Vitousek, 1990; Chapin and others, 1994; Adler and others, 1998).

It is evident even from the brief survey reported here that rhizosphere NA is relatively widespread among the plants inhabiting these peatlands as nearly 90% were NA positive. The data also suggest that this activity is common to peatlands in this region of the U.S. Since rhizosphere NA was not predictably associated with all signature peatland plants or consistently with the same species between peatlands, other factors (*e.g*., microscale nutrient heterogeneity) need to be considered during evaluation of nitrogen fixation or other important wetland functions. Diazotroph activity may represent a significant seasonal source of fixed nitrogen for the community (Henry and Svoboda, 1986; Quale, 1994; Waughman and Bellamy, 1980) and warrants further field and laboratory study.

Acknowledgments. We thank the Ohio Department of Natural Resources, Division of Natural Areas and Preserves for permission to conduct this study of Kent and Triangle Lake Bogs and Herrick Fen, and the Cleveland Museum of Natural History for permission to study Fern Lake Bog. We also acknowledge the financial support provided by the Department of Biological Sciences at Kent State University.

LITERATURE CITED

Adler, P. B., C. M. D'Antonio, and J. T.Tunison. 1998. Understory succession following a dieback of *Myrica faya* in Hawai'i Volcanoes National Park. Pacific Science 52:69-78.

Andreas, B. K. 1985. The relationship between Ohio peatland distribution and buried river valleys. Ohio Journal of Science 85:116-125.

Andreas, B. K. and G. R. Bryan. 1990. The vegetation of three *Sphagnum*-dominated basin-type bogs in northeastern Ohio. Ohio Journal of Science 90:54-66.

Andreas, B. K. and J. D. Knoop. 1992. 100 years of change in Ohio peatlands. Ohio Journal of Science 92:130-138.

Baker, D. D. and B. C. Mullin. 1992. Actinorhizal symbioses. p. 259-292. In: G. Stacey, R.H. Burris and H.J. Evans (eds.) Biological Nitrogen Fixation. Chapman & Hall, New York, NY, USA.

Baldani, J. I., A. L. M. Oliveira, S. L. Guimarães, V. L. D. Baldani, F. B. Reis Jr., L. G. Silva, V. M. Reis, K. R. S. Teixeira and J. Döbereiner. 2000. Biological nitrogen fixation (BNF) in non-leguminous plants: The role of endophyhtic diazotrophs. p. 397-400. In: F. O. Pedrosa, M. Hungria, M. G. Yates and W. E. Newton (eds.) Nitrogen Fixation: From Molecules to Crop Productivity. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Beaupied, H., A. Moiroud, A. M. Domenach, F. Kurdalif and R. Lensi. 1990. Ratio of fixed and assimilated nitrogen in a black alder *Alnus glutinosa* stand. Canadian Journal of Forest Research 20:1116-1119.

Biesboer, D. D. 1984. Nitrogen fixation associated with natural and cultivated stands of *Typha latifolia* L. (Typhacea). American Journal of Botany 71:505-511.

Capone, D. G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. p. 621-631. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr and J. J. Cole (eds.) Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, USA.

Chapin, F. S., L. R. Walker, C. L. Fastie and L. C. Sharman. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. Ecological Monographs 64:149-175.

Dean, J. V. and D. D. Biesboer. 1986. The uptake and distribution of $^{15}N_2$ into the various organs of *Typha latifolia* L. Aquatic Botany 23:309-320.

Del Tredici, P. 1995. A nitrogen fixation: the story of the *Frankia* symbiosis. Arnoldia (Jamaica Plain) 55:26-31.

Denny, G. L. 1979. Bogs. p. 141-150. In: M. B. Lafferty (ed.) Ohio's Natural Heritage. The Ohio Academy of Science, Columbus, OH, USA.

Dommergues, Y. R. 1997. Contribution of actinorhizal plants to tropical soil productivity and rehabilitation. Soil Biology and Biochemistry 29:931-941.

Dudley, J. L., B. Michener and K. Lajtha. 1996. The contributions of nitrogen-fixing symbioses to coastal heathland succession. American Midland Naturalist 135:334-342.

Eady, R. R. 1992. The dinitrogen-fixing bacteia. p. 534-553. In: A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K. H. Schleifer (eds.) The Prokaryotes, 2e, vol. III. Springer-Verlag, New York, NY, USA.

Eckardt, N. A. and D. D. Biesboer. 1988a. A survey of nitrogen fixation (acetylene reduction) associated with *Typha* in Minnesota. Canadian Journal of Botany 66:2419-2423.

Eckardt, N. A. and D. D. Biesboer. 1988b. Ecological aspects of nitrogen fixation (acetylene reduction) associated with plants of a Minnesota wetland community. Canadian Journal of Botany 66:1359-1363.

Ekblad, A. and K. Huss-Danell. 1995. Nitrogen fixation by *Alnus incana* and nitrogen transfer from *A. incana* to *Pinus sylvestris* influenced by macronutrients and ectomycorrhiza. New Phytologist 131:453-459.

Elkan, G. H. and C. R. Bunn. 1992. The rhizobia. p. 2197-2213. In: A. Balows, H. G. Trüper, M. Dworkin, W. Harder and K. H. Schleifer (eds.) The Prokaryotes, 2e, vol. III. Springer-Verlag, New York, NY, USA.

Elmerich, C., W. Zimmer and C. Vieille. 1992. Associative nitrogen-fixing bacteria. p. 212-258. In: G. Stacey, R. H. Burris and H. J. Evans (eds.) Biological Nitrogen Fixation. Chapman & Hall, New York, NY, USA.

Evans, H. J. and R. H. Burris. 1992. Highlights in biological nitrogen fixation during the last 50 years. p. 1-42. In: G. Stacey, R. H. Burris and H. J. Evans (eds.) Biological Nitrogen Fixation. Chapman & Hall, New York, NY, USA.

Franco, A. A., E. F. C. Campello, S. M. de Faria and L. E. Dias. 2000. The importance of biological nitrogen fixation on land rehabilitation. p. 569-570. In: F.O. Pedrosa, M. Hungria, M. G. Yates and W. E. Newton (eds.) Nitrogen Fixation: From Molecules to Crop Productivity. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Henry, G. H. R. and J. Svoboda. 1986. Dinitrogen fixation (acetylene reduction) in high arctic sedge meadow communities. Arctic and Alpine Research 18:181-187.

Kana, T. M. and J. D. Tjepkema. 1978. Nitrogen fixation associated with *Scirpus atrovirens* and other nonnodulated plants in Massachusetts. Canadian Journal of Botany 56:2636-2640.

Klucas, R. V. 1991. Associative nitrogen fixation in plants. p. 187-198. In: M. J. Dilworth and A. R. Glenn (eds.) Biology and Biochemistry of Nitrogen Fixation. Elsevier, New York, NY, USA.

Koerselman, W., H. De Caluwe and W.M. Kieskamp. 1989. Denitrification and dinitrogen fixation in two quaking fens in the Vechtplassen area, The Netherlands. Biogeochemistry 8:153-165.

Li, R. and I. C. MacRae. 1992. Isolation and identification of N_2-fixing enterobacteria associated with sugarcane in tropical Australia. Journal of General and Applied Microbiology 38:523-531.

Maeda, K., Y. Yamamura, H. Ozawa and Y. Hori. 1999. A comparison of photosynthetic capacity between *Myrica gale* var. *tomentosa*, a nitrogen-fixing shrub, and co-occurring species in an oligotophic moor. Photosynthetica (Prague) 36:11-20.

Miller, R. E., D. L. Reukema and T. A. Max. 1993. Size of Douglas-fir trees in relation to distance from a mixed red alder-Douglas-fir stand. Canadian Journal of Forest Research 23:2413-2418.

Ogan, M. 1984. Characteristics and nitrogenase activity (acetylene reduction) of microaerophilic bacteria from roots of aquatic angiosperms. Canadian Journal of Microbiology 30:955-960.

Ogan, M.T. 1982. Seasonal changes in rates of nitrogen-fixation (AR) and numbers of potential nitrogen-fixing bacteria associated with excised roots of aquatic angiosperms. Aquatic Botany 12:257-276.

Quale, R. K. 1994. Nitrogen fixation and denitrification in a constructed fen and comparisons with a natural fen in southwestern Ohio. M.S. Thesis. Wright State University, Dayton, OH, USA.

Ronkko, R., A. Smolander, E.-L. Nurmiaho-Lassila and K. Haahtela. 1993. *Frankia* in the rhizosphere of nonhost plants: A comparison with root-associated nitrogen-fixing *Enterobacter*, *Klebsiella* and *Pseudomonas.* Plant and Soil 153:85-95.

Sheridan, R. P. 1991. Nitrogenase activity by *Hapalosiphon flexuosus* associated with *Sphagnum erythrocalyx* mats in the cloud forest on the volcano La Soufrière, Guadeloupe, Fench West Indies. Biotropica 23:134-140.

Tjepkema, J. D. and H. J. Evans. 1976. Nitrogen fixation associated with *Juncus balticus* and other plants of Oregon wetlands. Soil Biology and Biochemistry 8:505-509.

Troelstra, S. R., R. Wagenaar and W. Smant. 1992. Growth of actinorhizal plants as influenced by the form of nitrogen with special reference to *Myrica gale* and *Alnus incana*. Journal of Experimental Botany 43:1349-1359.

Uselman, S. M., R. G. Qualls and R. B. Thomas. 1999. A test of a potential short cut in the nitrogen cycle: The role of exudation of symbiotically fixed nitrogen from the roots of a N-fixing tree and the effects of increased atmospheric CO_2 and temperature. Plant and Soil 210:21-32.

Vitousek, P. M. 1990. Biological invasions and ecosystem processes towards an integration of population biology and ecosystem studies. Oikos 57:7-13.

Voss, E. G. 1972. Michigan Flora. Cranbrook Institute of Science, Bloomfield Hills, MI, USA.

Wagner, G. M. and K. E. Mshigeni. 1986. The *Utrichularia*-Cyanophyta association and its nitrogen-fixing capacity. Hydrobiologia 141:255-261.

Walker, L. R. 1989. Soil nitrogen changes during primary succession on a floodplain in Alaska USA. Arctic and Alpine Research 21:341-349.

Waughman, G. J. and D. J. Bellamy. 1980. Nitrogen fixation and the nitrogen balance in peatland ecosystems. Ecology 61:1185-1198.

Wickstrom, C. E. and J. L. Corkran. 1997. Nitrogenase activities associated with macrophytes from a lacustrine and a freshwater estuarine habitat. Aquatic Botany 59:157-162.

Young, D. R., E. Sande and G. A. Peters. 1992. Spatial relationships of *Frankia* and *Myrica cerifera* on a Virginia, USA barrier island. Symbiosis12:209-220.

Zechmeister-Boltenstern, S. and H. Kinzel. 1990. Non-symbiotic nitrogen fixation associated with temperate soils in relation to soil properties and vegetation. Soil Biology and Biochemistry 22:1075-1084.